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Effects of electrical stimulation of the mammillary bodies on autonomic and regulatory functions in the anesthetized cat.

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EFFECTS OF ELECTRICAL STIMULATION OF THE MAMMILLARY BODIES ON AUTONOMIC AND REGULATORY FUNCTIONS IN THE ANESTHETIZED CAT

A Thesis Presented

By

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Finally, I would like to thank my cats for donating their beautiful brains.

ABSTRACT

Autonomic responses were recorded from five female cats during electrical stimulation of the medial or lateral mammillary nucleus and surrounding hypothalamic areas. In three of the five cats, the only notable responses at ventral levels within both nuclei was a slight pupillary dilatation, while in the remaining two animals slight changes in heartrate, blood pressure and respiration could be detected. In all cases, responses elicited from MMN or LMN were greatly reduced over those obtained at more dorsal levels. Two possible interpretations are offered: 1) The slight autonomic responses elicited from areas within the mammillary capsule can be attributed to the spread of electric current to surrounding tissue controlling such responses, 2) The mammillary bodies can be viewed as having some autonomic functions which are secondary to their major role in a complex behavioral system.

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In the present study, an attempt will be made to determine the extent of involvement of the mammillary bodies in the autonomic functions of the posterior hypothalamus.

Anatomical description of the mammillary nuclei and surrounding structures

The mammillary bodies (MB) have traditionally been described as belonging to the diencephalic complex known as the hypothalamus and geographically at least, there is no doubt that this is the case. Appearing as well developed nuclei only in mammals, the MB's are found in the ventral portion and slightly caudal to the posterior hypothalamus on either side of the midline, each consisting of a large medial mammillary nucleus (MMN), a spherical complex composed of small to medium sized cells, and a smaller lateral mammillary nucleus (LMN), composed of slightly larger cells and lying directly lateral to the medial nucleus. The premammillary nuclei are small indistinct cell groupings, located lateral to the posterior periventricular area. These nuclei serve as the rostral boundary of the mammillary nuclei but are not considered part of the mammillary complex itself. Dorsally, the mammillary bodies are demarcated by the supramammillary nucleus, a small aggregate of cells lying immediately dorsal to MMN. Just dorsal to the mammillary bodies in caudal sections, the supramammillary decussation is visible on the midline. Also known as the hypothalamic or subthalamic commissure, this structure consists of a slender group of fibers which interconnects the subthalamic nucleus, red nucleus, midbrain tegmentum and globus pallidus of one side with their contralateral counterparts. In addition, the dorsal border of the mammillary bodies can be identified by a band of heavily myelinated fibers encasing MMN which form the mammillary capsule.

The major afferents to MMN include the fornix and the median forebrain bundle, both descending fiber tracts from forebrain areas, and the mammillary peduncle, an ascending tract carrying fibers from the tegmentum. The efferents of MMN include three bundles, all of which leave the mammillary bodies via the principal mammillary tract before branching to form the descending mammillotegmental tract which terminates in the limbic midbrain area of Nauta, the mammillothalamic tract (MTT) coursing mainly to the anteroventral and anteromedial nuclei (ATN) as well as to the ventromedial nuclei of the thalamus, and the lateral offset whose fibers terminate diffusely in the subthalamus. The afferent and efferent connections of LMN are very similar to those of MMN, with the most important distinction for the purpose of this study being that fibers of the MTT ascending to the thalamus from the lateral nucleus project mainly to the anterodorsal nucleus both ipsilaterally and contralaterally rather than terminating in ATN.

Functions of the mammillary bodies

The hypothalamus has been unquestionably shown to be involved with homeostatic mechanisms including sexual behavior, feeding, drinking, autonomic and thermoregulatory functioning, etc. As a result of the close geographical location of the mammillary bodies, this description has often been generalized to include them in spite of a lack of compelling independent evidence in many cases. Numerous studies involving lesions or stimulation in hypothalamic and midbrain areas have cited the MB's as serving certain regulatory functions such as those described above. Sawyer (1956, 1959) reported that lesions in the MB's of rabbits produced permanent anestrus and suggested also that the afferent projections to the mammillary nuclei from the olfactory bulb via the median forebrain bundle and from hippocampus by way of the fornix, and possibly also the efferent projections through the MTT may be influenced by estrogen to evoke normal mating behavior, although these structures may not be essential for ovulation.

Porter (1952) claimed a marked increase in the frequency and amplitude of electrical activity in the posterior hypothalamus of cats in response to stress as measured by the level of circulating eosinophils in the blood following injections of epinephrine and insulin.

In a later study using monkeys, Porter (1954) found that lesions of the posterior hypothalamus (including the MB's) destroyed the eosinopenic response to stress stimuli and that when no lesions were present, direct stimulation of certain hypothalamic regions which again supposedly included the MB's, produced definite depressions in the level of circulating eosinophils even without the injection of a stressing agent. Porter was probably one of the earliest investigators to suggest that the posterior hypothalamic firing may in fact represent an autonomic discharge in the CNS which effects secretion of adrenocorticotrophic hormone (ACTH) in the adenohypophysis.

In more recent studies, Cragg has attempted to relate not only the mammillary bodies themselves but also their major afferent connections to changes in autonomic responding. In one experiment (Cragg, 1958) electrical stimulation of the ventral portion of the fimbria resulted in an increase in blood pressure and the rate and amplitude of respiration. The fimbria comprises the main efferent tract from the hippocampus and is known to contain fibers terminating in the septum and the MMN. In a later study (Cragg and Hamlyn, 1959), electrical stimulation of the dorsal fornix evoked an electrical response in the MMN and repetitive stimulation in the same area was found to lower blood pressure, increase respiration and reduce the knee-jerk reflex. These authors also report that similar responses were elicited from the lateral septal nucleus and MMN.

It should be noted that none of the above studies provide detailed descriptions of the histological techniques utilized in determining the location and extent of lesions or in the verification of electrode tracts.

On the other hand, other studies have demonstrated a specific absence of MB involvement in autonomic and regulatory functioning. Ranson and Magoun (1939) demonstrated a marked increase in autonomic activity in cats, as measured by the depth and rate of respiration, as a result of electrical stimulation of the supramammillary decussation and the periventricular fibers. The MB's specifically, however, were reported not to have elicited the response with the exception of a few points along the dorsolateral border. At the level of the caudal border of the MB's augmented responses were also obtained from the lateral hypothalamic area and from the region dorsolateral to the MB's.

Akert and Andy (1955) also report no changes in respiration and intrafemoral blood pressure following electrical stimulation in the region of the mammillary bodies in the cat.

In spite of the work of Cragg and others implicating the MB's and related structures in autonomic behavior, it is actually the anatomical connections of the MB's which suggest most strongly that this system is probably not autonomic in function. The MTT, the major efferent projection of the mammillary nuclei has already been discussed. Information arriving at the anterior thalamus via this tract is projected directly to the cingulate gyrus and entorhinal cortex and finally arrives at the hippocampal formation. From areas CA 1 and the anterior portion of CA 2 of the hippocampus, fornix fibers complete the circuit to the MB's. This circuit was originally described by Papez in 1939 as the possible neurological substrate of emotion, a characteristic rarely attributed to premammalian species. The fact that this system appears so highly developed in mammals, particularly man, while being virtually nonexistent in birds and reptiles (see Krieckhaus and Wagman, 1967 and Krieckhaus, 1967), seems to indicate a level of functioning which must be much more recent phylogenetically than the homeostatic mechanisms known to be mediated by the hypothalamus in all classes of vertebrates. Thus, it appears that although the mammillary bodies may have originally been derived from hypothalamic tissue, the projections that have developed through phylogeny clearly suggest that their function has taken a significantly different direction.

In the area of human brain research, there have been numerous attempts to relate the destruction of the mammillary bodies to Korsakoff's syndrome. This disorder, first described in 1889, may include such symptoms as disorientation to immediate surroundings along with acute anterograde amnesia. In many cases, memory of many past events does not appear to be severely effected. Originally, the disease was believed to involve cortical damage, but later the pathological tissue was said to be localized in the periventricular region of the thalamus and hypothalamus which included the MB's (Gamper, 1928). Since that time, many studies have been devoted to supporting or refuting the notion that pathology of the mammillary bodies bears some causal relationship to this amnestic syndrome (see Krieckhaus, 1962). Although many cases have illustrated memory distortions and other symptoms of the Korsakoff psychoses in patients later found to show severely degenerated tissue in and around the mammillary bodies, the evidence is inconclusive and any attempt to define a discrete relationship between these structures and the disease would certainly be unwarranted at the present time.

The work of Krieckhaus and his co-workers over the past ten years offers the only significant behavioral data which attempts to relate the mammillary bodies and the mammillothalamic system to a function which is not autonomic in nature. Krieckhaus has found through repeated studies (1962-1967), that transsection of the MTT in cats and rats leads to a decrement in two-way avoidance behavior in which the animal is required to return repeatedly to a compartment where he has previously experienced a painful electric shock. Such a response would seem highly unlikely in the animal's natural habitat, yet normal animals can learn the task prior to lesions of the MTT. The possibility that the decrement following this lesion could simply be the result of a memory impairment has been ruled out by the fact that simple one-way avoidance is not affected by similar lesions and that escape behavior, when finally initiated in the two-way avoidance task, shows response latencies consistent with the animal's preoperative performance. It is as if the animal "knows" the correct response but the triggering mechanism necessary for it's initiation is missing. These findings have led to the hypothesis that the mammillothalamic system may provide a sort of "triggering" function necessary for the initiation of behaviors which are low in the normal response hierarchy of the animal, or behaviors for which the animal may be said to be "contraprepared" (see Seligman, 1970). This ability to respond independently to unique environmental situations could be a highly adaptive characteristic and can be viewed as part of a more generalized trend toward less rigid and more "plastic" behavior patterns which become more prominent as one ascends the phylogenetic scale.

Unfortunately, the studies thus far conducted are far from offering conclusive evidence for the above hypothesis. One experiment which is critical to elucidating the function of the MB's and their role in the mammillothalamic system involves the electrical stimulation of precisely defined anatomical areas in and around the mammillary complex. Whether elicitation of autonomic responses is possible in the entire posterior hypothalamus as has been claimed in past stimulation studies or whether the MB's provide a unique exception is the question which this study addresses. Although the present investigation will not provide behavioral data in support of the hypothesis of Krieckhaus and his associates, the demonstration that the MB's themselves are not involved in directly mediating autonomic functions would certainly renew the question of the role played by these prominent structures in mammalian behavior and leave open the interpretation of Krieckhaus for further investigation. On the other hand, if discrete stimulation of the MB's is found to elicit autonomic responses, a reinterpretation of the above mentioned behavioral data will indeed be called for.

METHODS

The subjects, female cats approximately 2 to 4 kg, were prepared for surgery by an intraperitoneal injection of sodium pentobarbitol at a dosage of approximately 35 mg anesthesia to 1 kg of body weight. Polyethylene tubing was inserted into the femoral vein for the injection of 1 cc of a nutritive glucose-ringers solution approximately every 2 hours and also for the injection of additional anesthesia if needed. The femoral artery was also cannulated to measure blood pressure through a pressure transducer. A tracheotomy was performed and a glass Y tube inserted for use in applying artificial respiration if needed, and provided for convenient placement of a thermister, for measuring respiration. The cat was then secured in a Kopf heavy duty stereotaxic apparatus with the body elevated to the level of the head to improve circulation. Heartrate was measured by means of two hypodermic needles inserted subcutaneously on either side of the thorax and the animal's temperature was maintained at 36-37 degrees C. by hot water bottles wrapped in towels. Blood pressure, respiration, and heartrate responses were all amplified and recorded on a Grass Model 5D polygraph.

The skull was opened with a high speed drill and rongeurs and the dura slit and resected, exposing a small portion of cortex. Concentric, bipolar electrodes, the outer barrel of teflon-insulated 27 gauge stainless steel hypodermic tubing and the inner wire consisting of 5 mil stainless steel diamel-coated wire extending 1.0 mm beyond the tip of the barrel were used in stimulation. The tip of the barrel and also the tip of the inside wire were bared of insulation for .5 mm. The electrodes were lowered unilaterally into the mammillary bodies, and in some animals into the anteroventral and anteromedial nuclei of the thalamus, and the hippocampus to assess any possible responses from these structures. When more than one electrode was placed, separate electrode carriers were used to allow for independent penetration. The number and order of stimulations for each structure varied depending on the responses obtained and will be discussed for each animal individually later.

The stimulus source was a Grass S88 square wave generator which delivered trains of monophasic cathodal pulses to the preparation via a Grass Stimulus Isolation Unit (SIU) and a Grass Constant Current Unit (CCU). Each stimulus train lasted no more than 3 seconds and consisted of 100 to 300 pps, the duration of each pulse from 0.1 to 0.5 ms. The current was maintained at around 1 ma and in no case did it exceed 3 ma. Voltage and current were continually monitored on a Hewlett-Packard 132 dual beam oscilloscope. In addition to the autonomic responses being recorded on the polygraph, pupillary dilatation, pilo-erection, and any observable bodily movements were carefully noted.

Following the completion of all stimulations, each cat was sacrificed under deep nembutal anesthesia by pericardial perfusion with physiological saline followed by a 10% formalin solution. The brain was removed and stored in 10% formalin-sucrose solution for several days. Frozen sections, 40 microns thick were cut and every section in the vicinity of an electrode tract was saved. Every fourth section was then mounted and stained with a cresyl violet cell stain and carefully examined under the microscope to determine the position of the electrode tip at maximum penetration.

RESULTS

Electrode placement within some portion of the mammillary bodies was achieved in five cats; in four the tip penetrated MMN while in the remaining cat the electrode was in LMN.

Although the base rate of the recorded responses differed for each animal, relative increases and decreases occuring at similar anatomical locations were quite consistent across subjects. A sample of the recorded responses of one animal ("Wise, Jr.") is presented in figures 1 and 2. Figure 1 shows a typical stimulation within the mammillary capsule, the electrode tip penetrating MMN. No notable changes in the responses occur following stimulation. In contrast, the responses of figure 2 were elicited from tissue at approximately the level of the supramammillary decussation.

Deviation scores for the respiratory response were calculated by measuring the amplitude (in cm) and the duration (in sec.) of a single inspiration at the point of greatest response following a stimulation and comparing these values to the average amplitude and duration of inspirations recorded prior to stimulation. Figures 3 through 12 show the deviation scores for all stimulations between a point approximately 3 mm dorsal to the MB's and the deepest point of penetration within the mammillary capsule. Both duration and amplitude scores obtained from stimulation of areas either dorsal to the MB's or within the mammillary capsule were compared for each cat using the Mann-Whitney U test for independent samples. Six of the ten comparisons (deviation scores for amplitude and duration in each of 5 cats) showed a highly significant difference between the 2 groups (α=.001), while two tests were significant at the .01 and one at the .05 level. Only

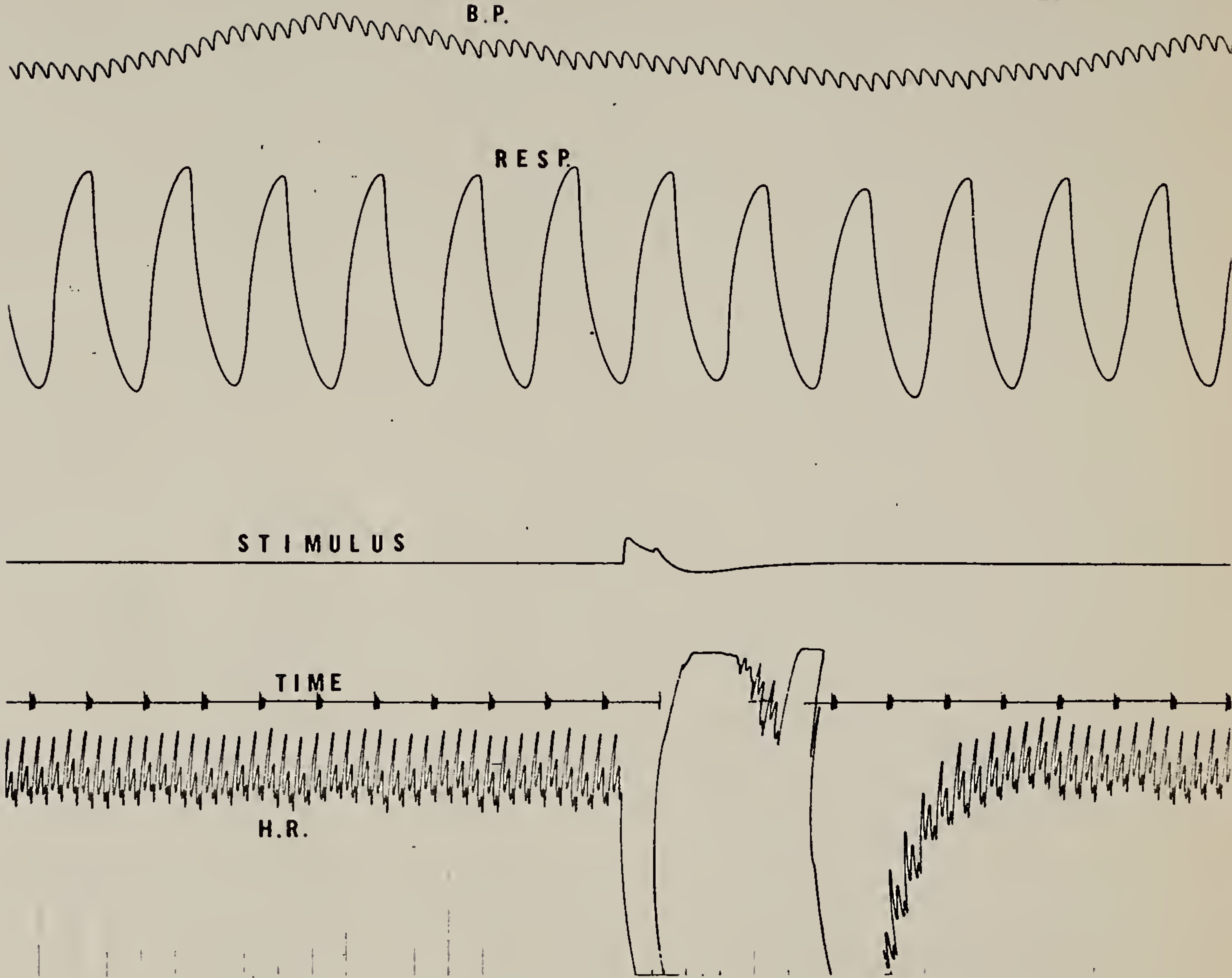

Fig. 1. Blood pressure, respiration and heartrate responses to stimulation of MMN. ("Wise, Jr.")

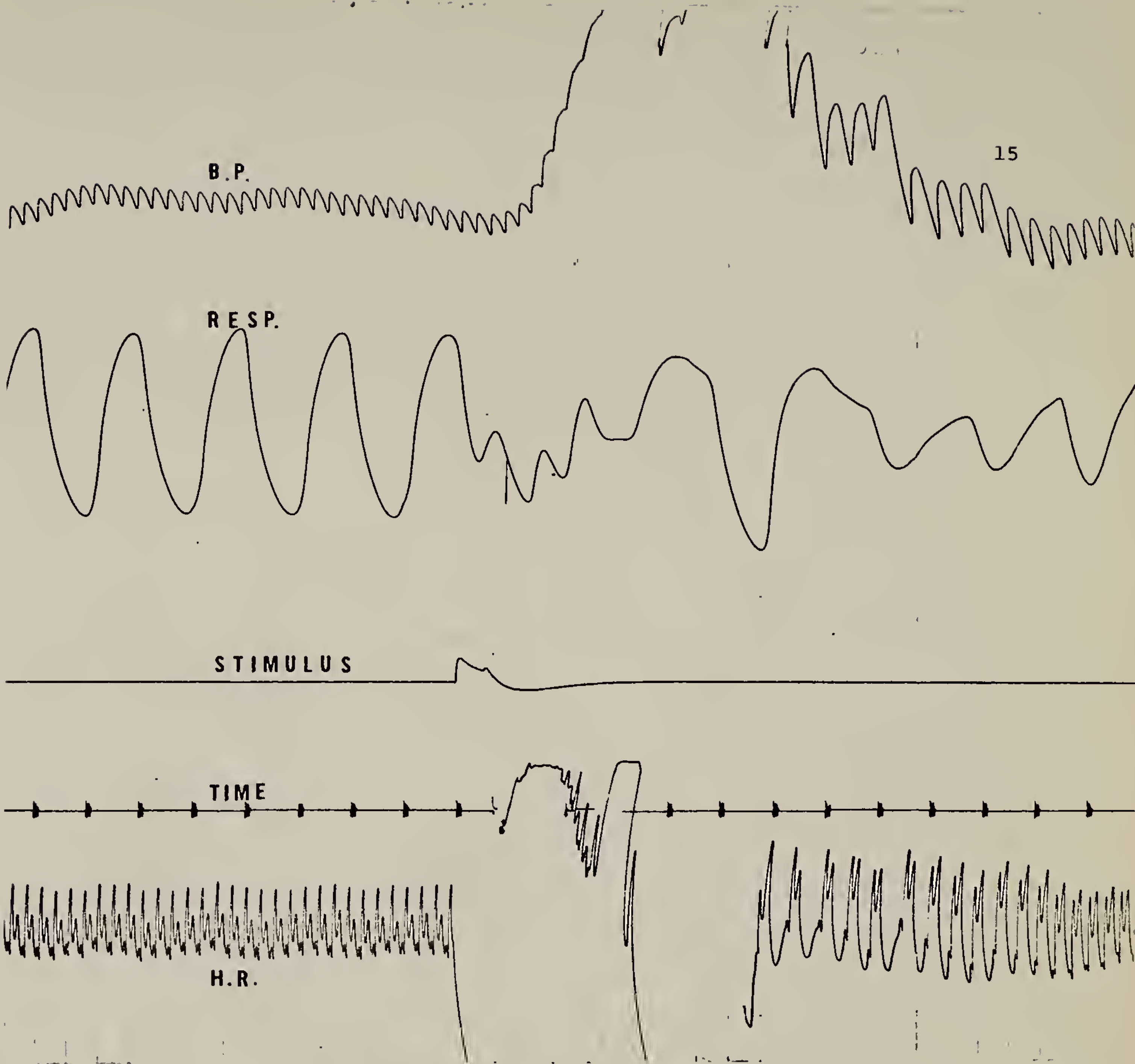

Fig. 2. Responses to stimulation in the area of the supra-mammillary decussation, about 2 mm dorsal to the border of the mammillary capsule. (Wise, Jr.")

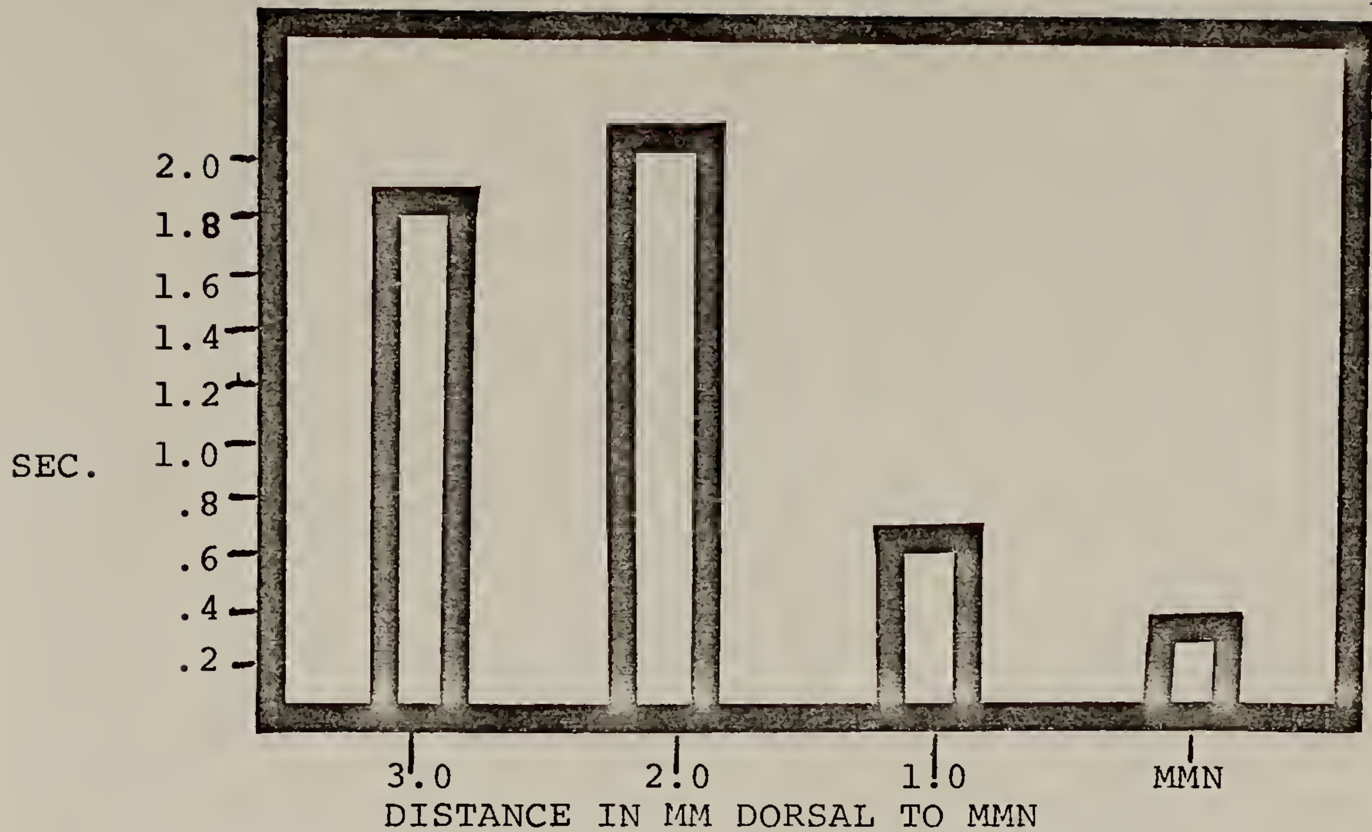

Fig. 3. Deviation values for duration of respiratory response at different levels of stimulation for "Scratchy".

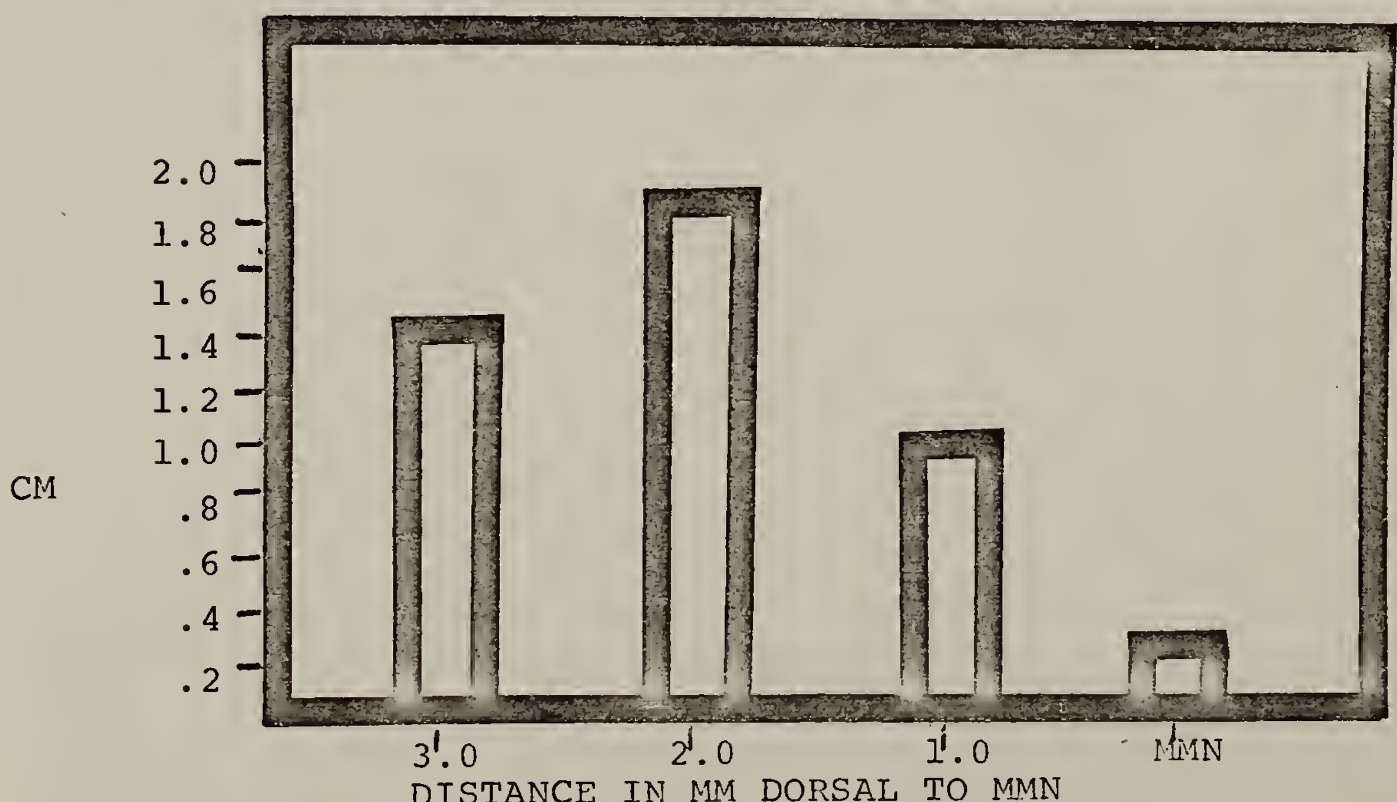

Fig. 4. Deviation values for amplitude of respiratory response at different levels of stimulation for "Scratchy".

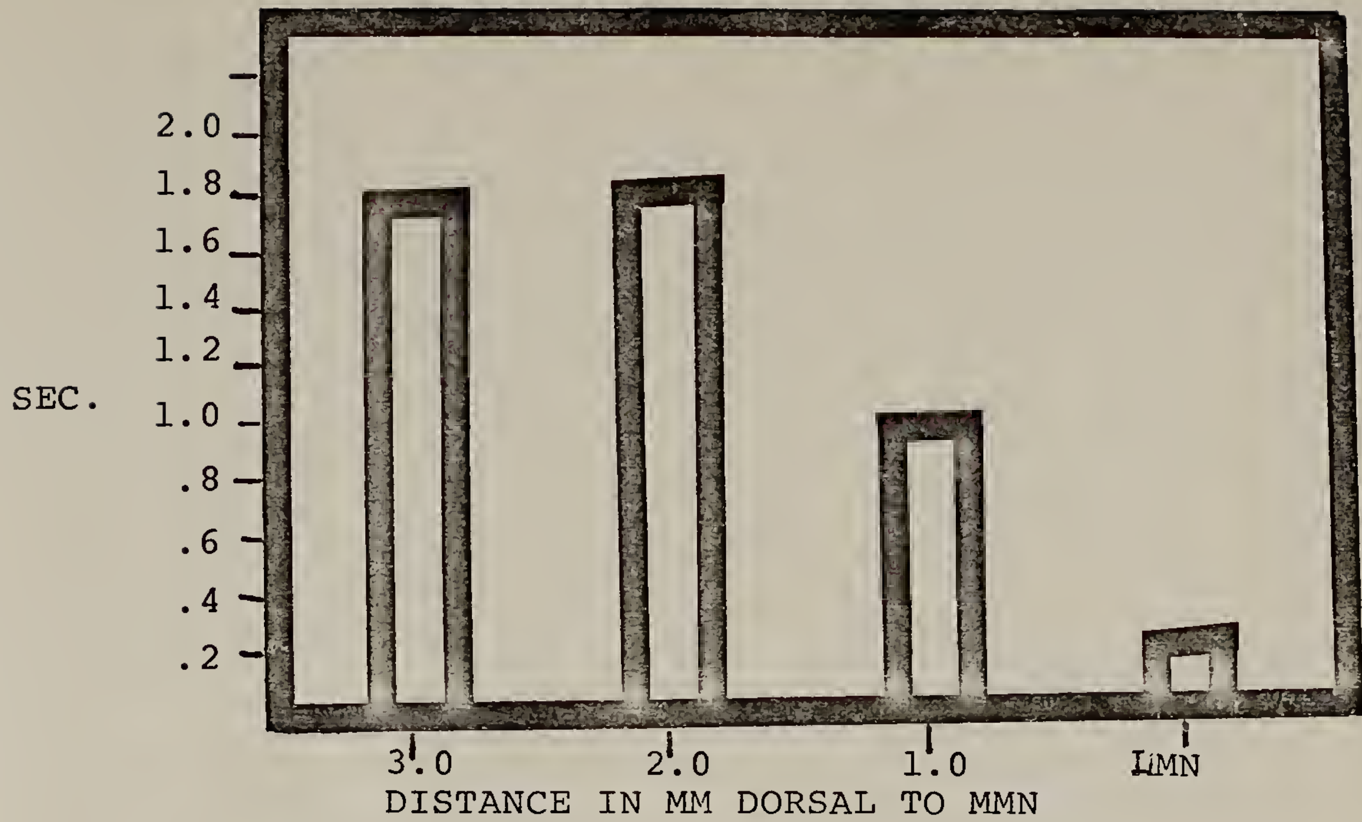

Fig. 5. Deviation values for duration of respiratory response at different levels of stimulation for "EEK".

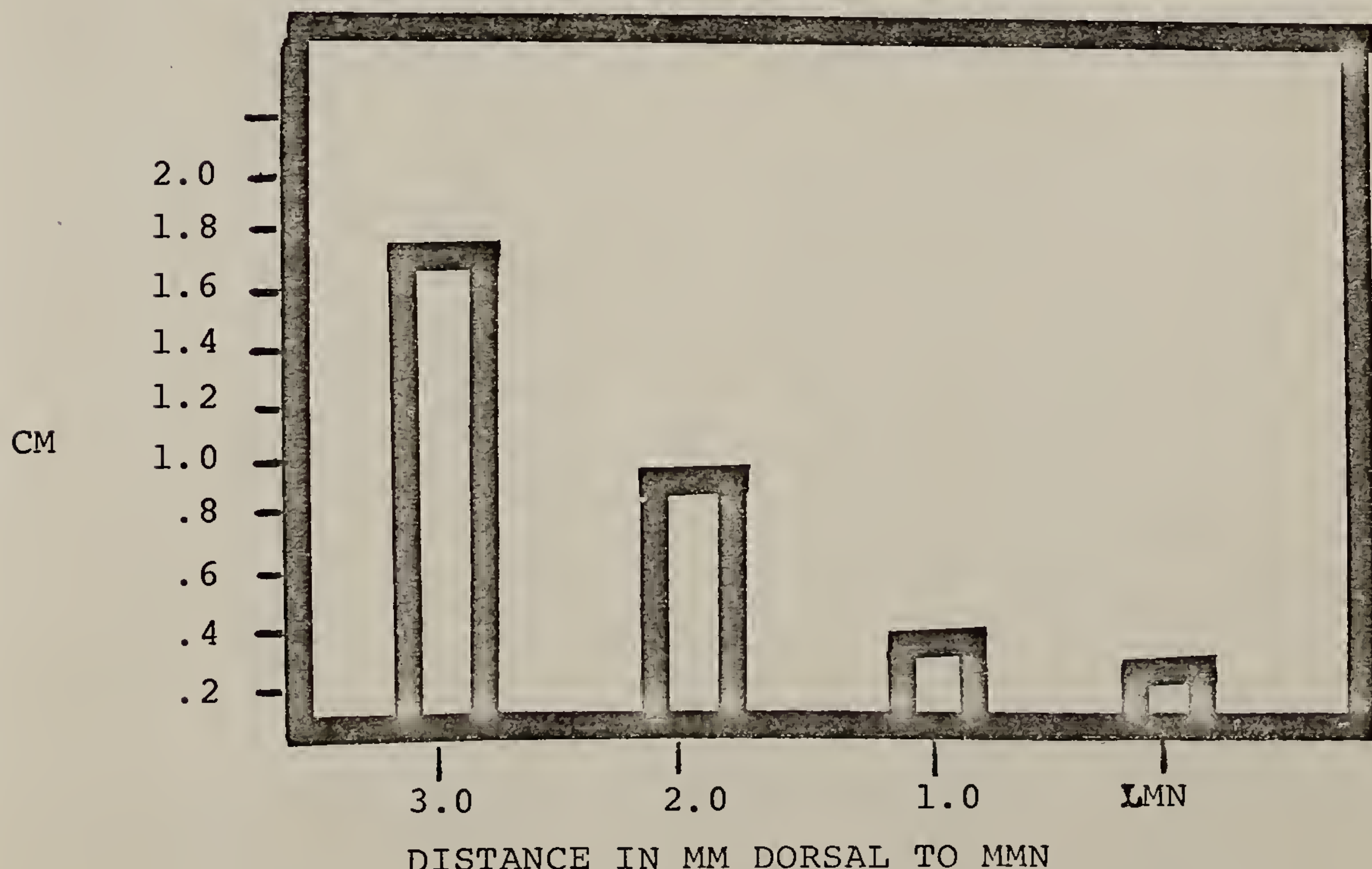

Fig. 6. Deviation values for amplitude of respiratory response at different levels of stimulation for "EEK".

Fig. 7. Deviation values for duration of respiratory response at different levels of stimulation for "Wise".

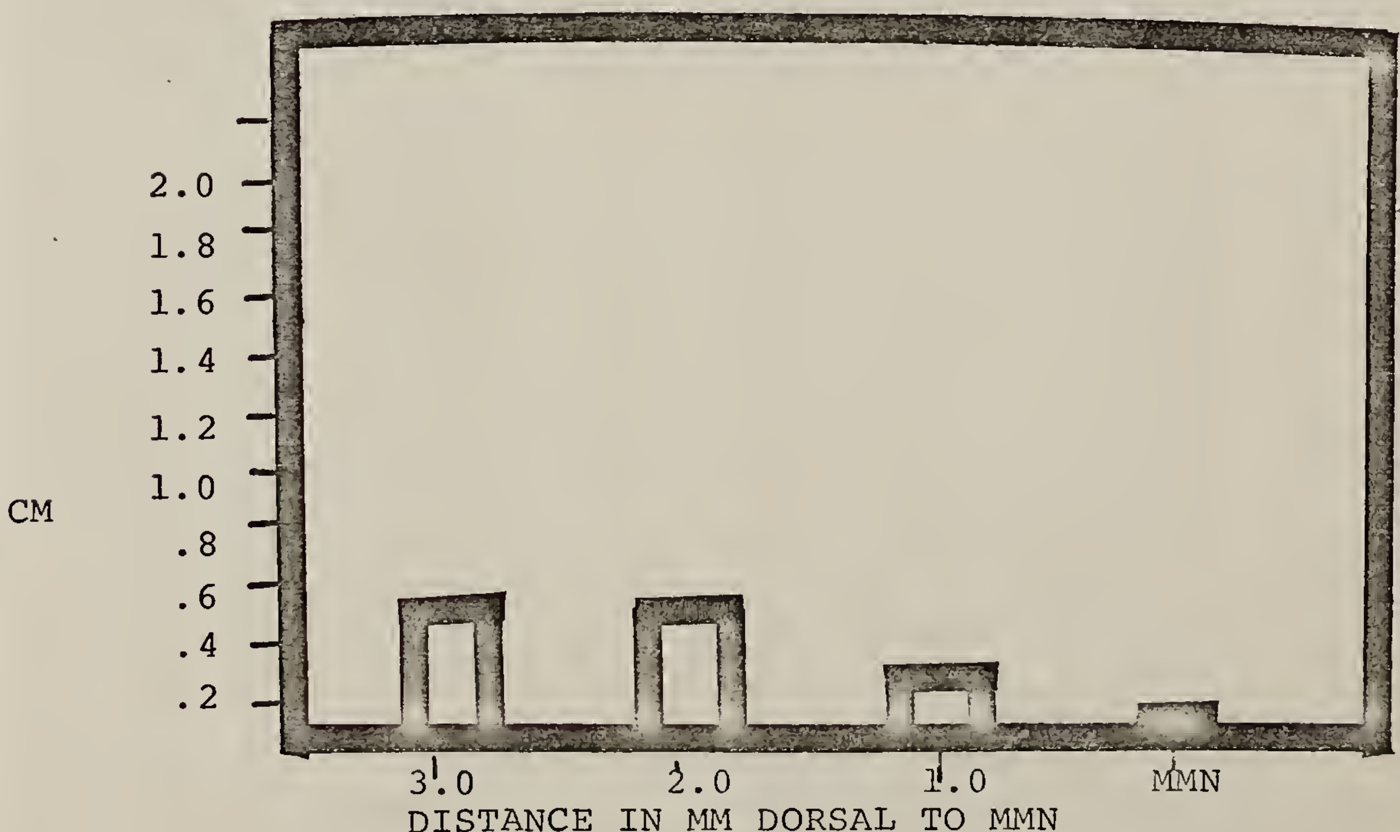

Fig. 8. Deviation values for amplitude of respiratory response at different levels of stimulation for "Wise".

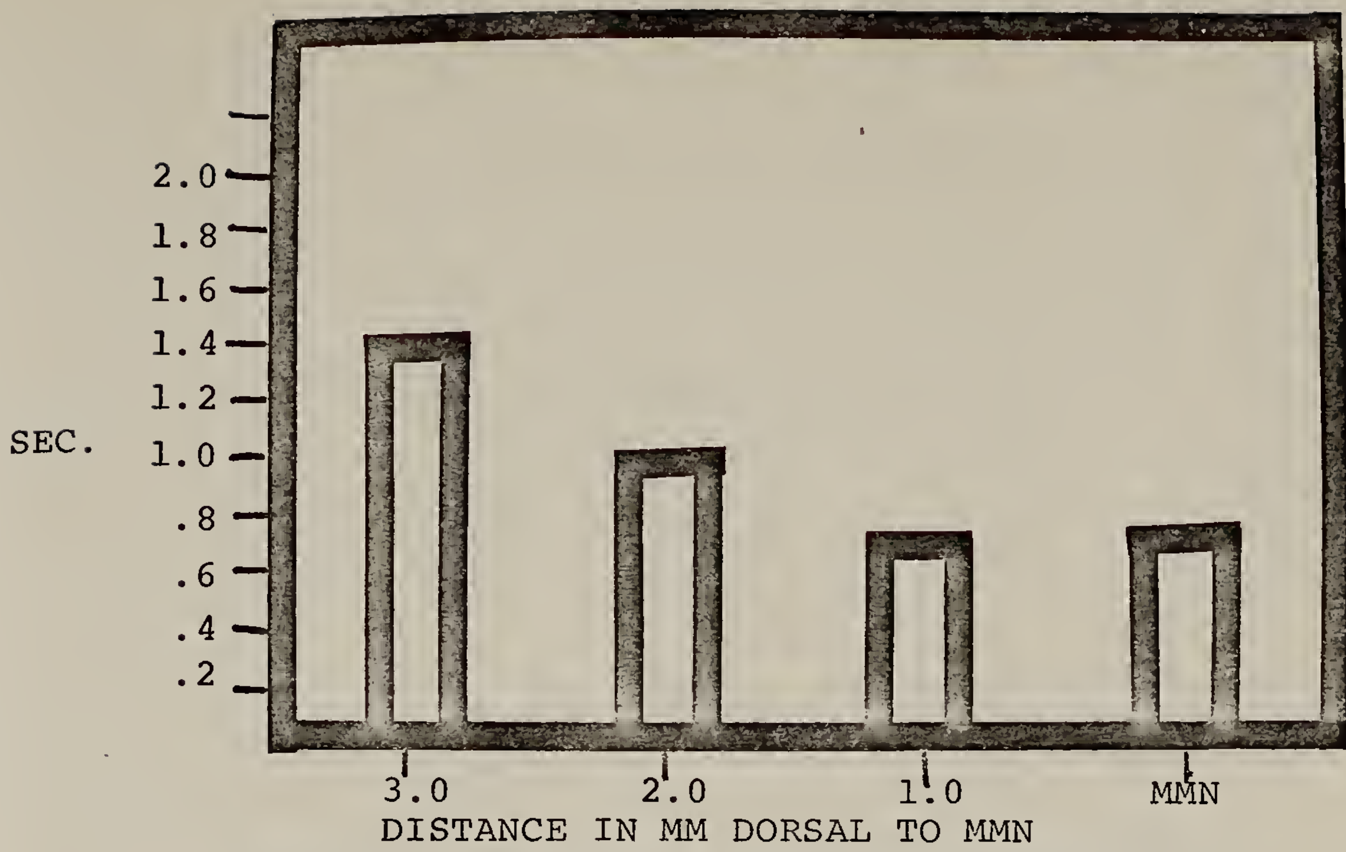

Fig. 9. Deviation values for duration of respiratory response at different levels of stimulation for "Simba".

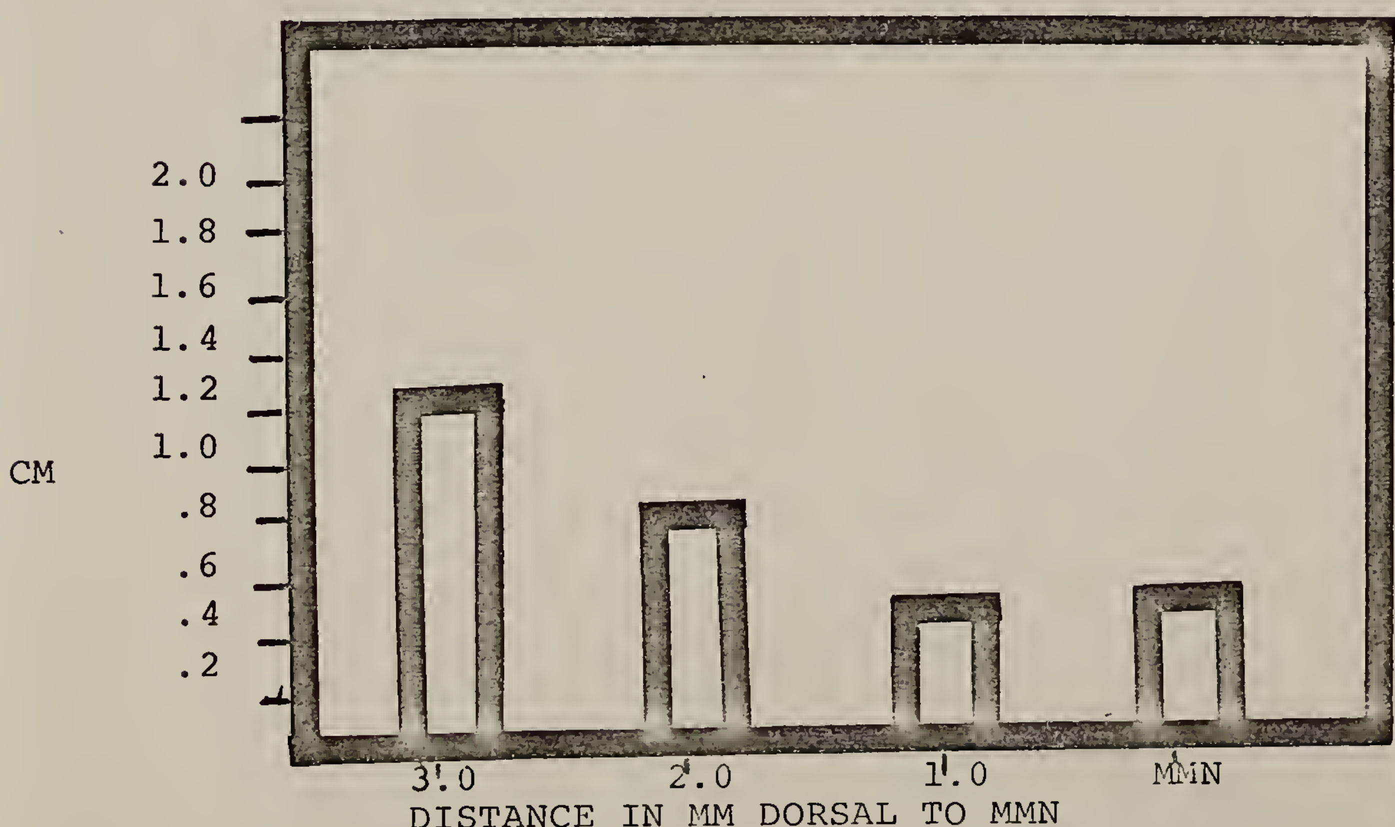

Fig. 10. Deviation values for amplitude of respiratory response at different levels of stimulation for "Simba".

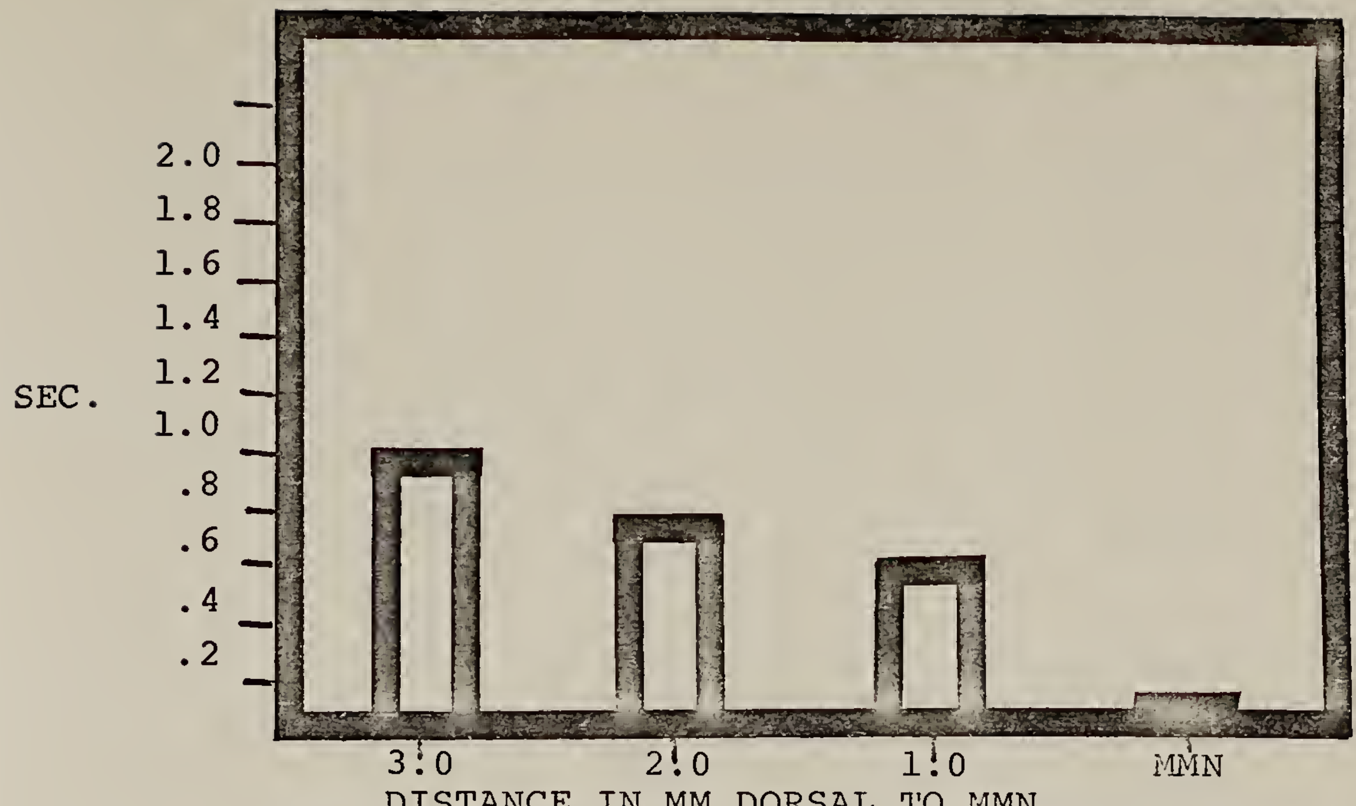

Fig. 11. Deviation values for duration of respiratory response at different levels of stimulation for "Wise, Jr.".

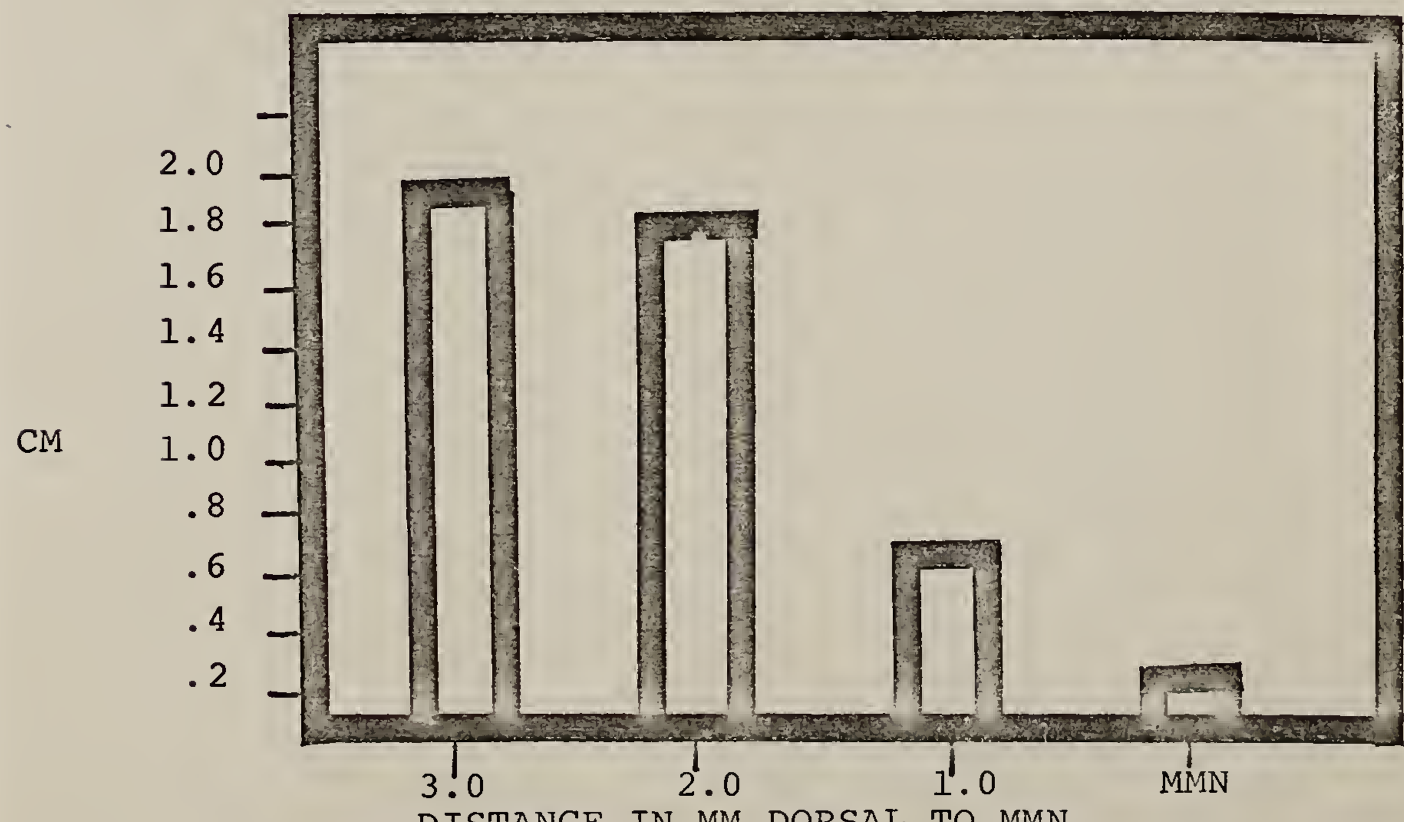

Fig. 12. Deviation values for amplitude of respiratory response at different levels of stimulation for "Wise, Jr.".

one comparison (the duration scores of "Simba") failed to show significance (see figure 9.).

Because of the relatively large A-P extent of the mammillary bodies (approximately 2 mm) and slight variations in the angle of electrode penetration, the anatomical loci associated with physiological responses will be discussed in detail for each animal. To aid in this description, the mammillary bodies have been arbitrarily divided into five sections from anterior to posterior and placement will be described as being for instance anterior, mid anterior, mid, mid posterior, and posterior for each animal. All anatomical descriptions have been histologically verified and are assumed accurate to within .5 mm based on a maximum of 10% shrinkage of the tissue during processing (see Krieckhaus, 1964b).

Unless otherwise indicated, the stimulus parameters did not vary from those values described under methods (p. 11). Current, continuously monitored on the CRO was usually 1 ma during initial stimulations at any particular loci, with any significant increases or decreases being specifically noted. Variations in electrode impedance were continuously calculated and in most cases found to be minimal. Impedance was always less than 10K ohms and will not be described for specific animals.

Description of responses at each level of stimulation for individual cats

"Scratchy"

In this cat, the electrode tip penetrated the posterior portion of the medial mammillary nucleus just off the midline and at the deepest point passed through the ventral extent of the MB's (see figure 13), but the tip was straight and in good condition on removal.

The first responses obtained from this animal occured upon stimulation of the left wall of the third ventricle and periventricular grey area 7 mm dorsal to the bottom of the brain. Responses included a moderate pupillary dilatation and an irregular respiratory pattern which lasted for approximately 7 sec following stimulus onset, in which breaths became shallow, uneven, and somewhat more rapid. In addition, there was a mild decrease in heartrate immediately after stimulus onset followed by a brief period in which the EKG appeared quite erratic. Three mm deeper, 0.5 mm dorsal to the supramammillary decussation, an increase in response strength occured with considerable muscular contractions of the limbs, a change in the respiratory pattern which now consisted of two shallow breaths at a slightly increased rate immediately following the stimulus and a moderate increase in B.P. EKG and pupillary dilatation remained unchanged. Little variation in this pattern

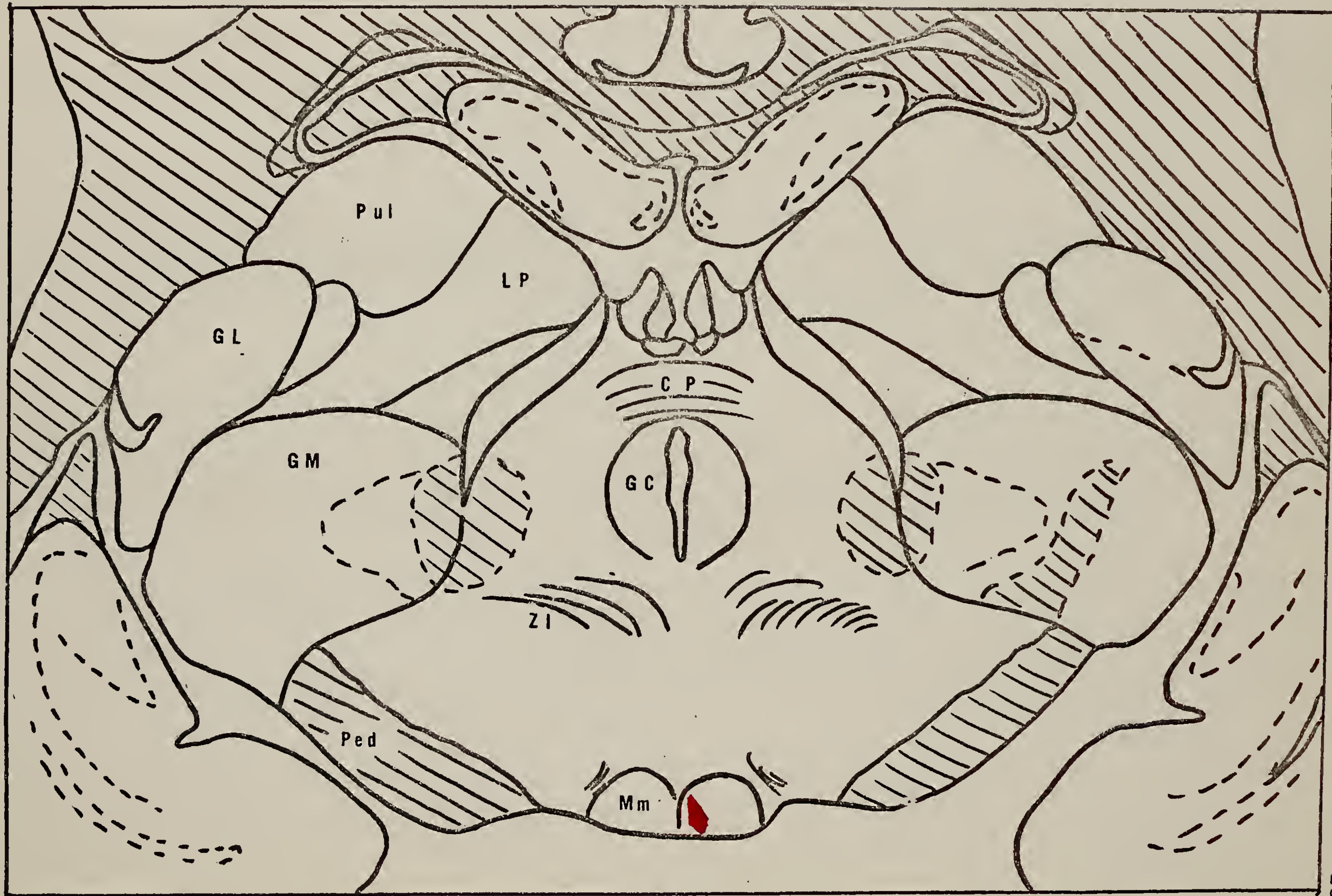

Fig. 13. "Scratchy" Position of electrode tip at deepest penetration.

was noted for the next 1.5 mm, until the tip finally reached the dorsal extent of MMN, at which point a marked decrease in all responses occured. Specifically, the respiratory response was reduced from the complex pattern described above to simply a mild increase in rate, the B.P. response was barely detectable as a slight increase, and in general, muscle contractions, piloerection, etc., were all greatly reduced. This attenuated response was found for the next 1 mm in MMN. At this level, responses included a mild to moderate pupillary dilatation and a somewhat inconsistent but generally slight respiratory response which usually took the form of one or more deep inspirations of a briefer duration and slightly increased rate than those observed under normal conditions. No changes in blood pressure or heartrate were recorded at this level. Further ventral, respiratory and pupillary responses were barely detectable and at deepest penetration (which is believed to have been ventral to the MB's by 0.5 mm at the bottom of the brain) no responses were observed during four consecutive stimulations using 1 ma of current.

Following initial penetration, the electrode was raised up to the level of the supramammillary decussation (the point which previously yielded the greatest response) and lowered again to MMN, repeating stimulations approx every 1 mm and the responses were similar to those recorded earlier at the same vertical levels. When the electrode was in MMN for the second time, the current was increased to 2 ma with no detectable increases in responses resulting. In addition to the responses previously described a new response pattern was elicited on the way up over a 1 mm area just dorsal to the deepest area stimulated. It consisted of a lateral eye movement usually accompanied by pupillary dilatation. The response pattern varied from one involving only the left eye to a full bilateral effect in which both eyes first moved medially, dilated, then moved laterally and constricted. The response was sharpened when the current was increased from 1 to 2 ma but was still only elicited from a restricted area in or near the most ventral portion of the MMN.

"<u>EEK</u>"

The electrode passed just lateral to MMN at approximately the mid A-P of the MMN nucleus. At deepest penetration, the tip rested in the center of LMN (see figure 14) and gradations of autonomic responses were recorded throughout a vertical extent of 7.5 mm dorsal to this point.

The first response observed, pupillary dilatation, was elicited by stimulation of tissue just lateral to

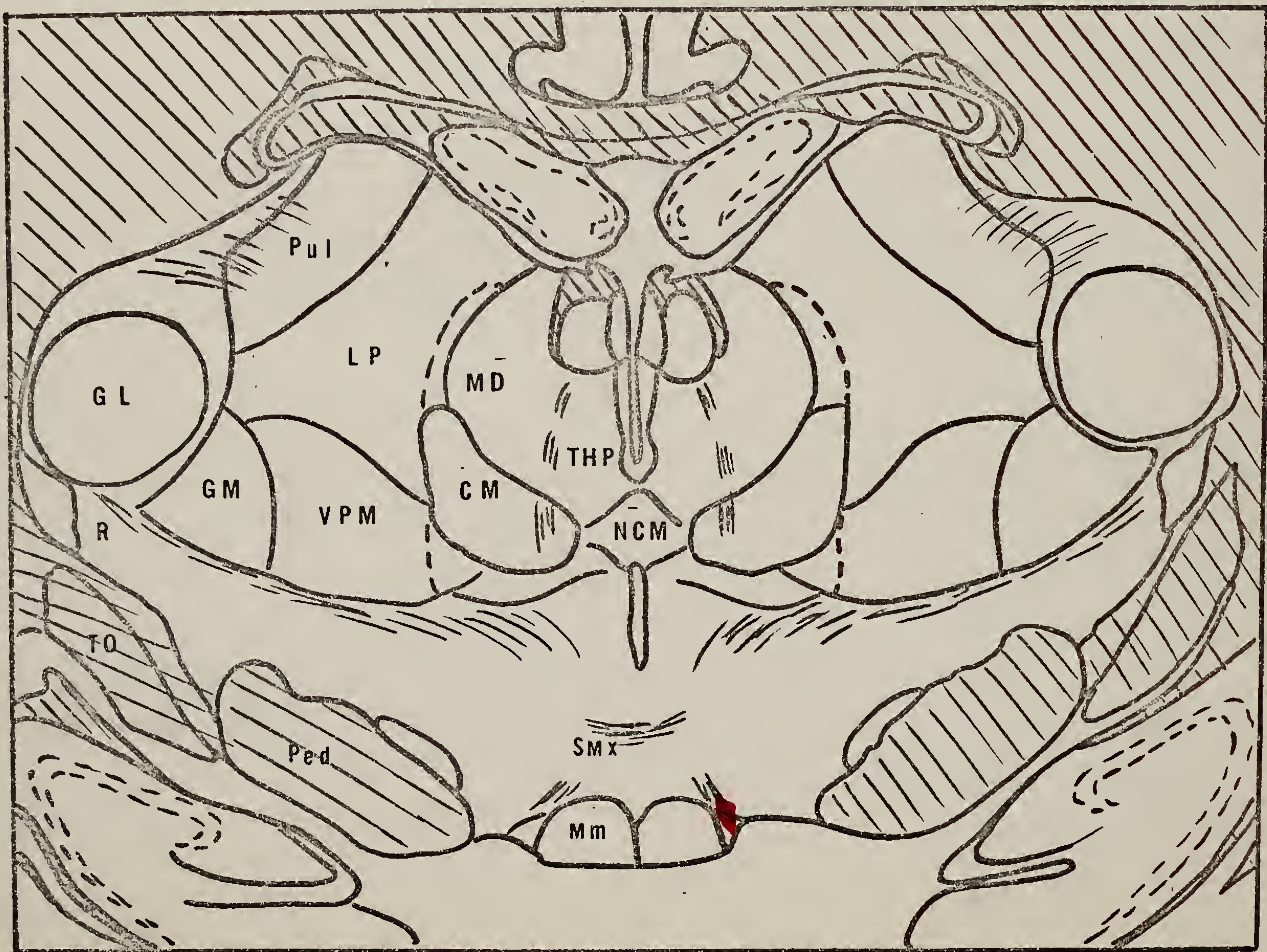

Fig. 14. "EEK" Position of electrode tip at deepest penetration.

the posterior portion of the central medial nucleus of the thalamus. No other responses were noted with the exception of a very slight increase in respiration. One mm deeper, in addition to the pupillary response, stimulation induced mild striate muscular responses and a deep inspiration with a latency of approximately 2 sec following stimulus onset. With continued stimulation at 1/2 mm steps, incremental increases in the strength of all autonomic responses were observed, the point of greatest response occuring about 1 mm lateral to the third ventricle and 3 mm dorsal to MMN at about the level of the supramammillary decussation and slightly anterior to the Fields of Forel. These responses included a moderate increase followed by a comparable decrease in blood pressure, a deep inspiration similar to that described above, an extremely irregular EKG pattern which outlasted the stimulus by about 10 sec, a full pupillary dilatation, piloerection, and strong muscular contractions, especially apparent in the limbs. Over an area 2 mm immediately ventral to this, similar responses were noted with the exception of a slight change in the respiratory pattern which occured at a point 2 mm dorsal to the MB's in the pre-rubral area. At the onset of the stimulus, the animal appeared to take 3 rapid shallow breaths followed by a deep inspiration as opposed to the deep inspiration following the stimulus which was noted earlier. This response persisted over a vertical area of 1 mm before declining.

At a point immediately dorsal to the MB's and approximately 2 mm off the midline (probably just inside the mammillary capsule) there occured a reduction in the strength of all responses except blood pressure which showed a larger increase and decrease than had been noted 2 mm above. The several shallow breaths of the respiratory pattern were replaced by one short inspiration followed by the usual deep inspiration which occured 3 sec after the stimulus onset. The EKG pattern also appeared less irregular and muscular contractions were not as obvious. One-half mm below this point, a further decrease in strength was apparent, the blood pressure response having decreased considerably from the point just dorsal and the respiratory response now having lost the deep inspiratory component.

When the electrode tip was just lateral to MMN and 1.5 mm dorsal to the point of deepest penetration there was a further decrease in response strength, the EKG and B.P. responses were barely detectable and respiration increased only very slightly. It was approximately at this level, however, that the pupillary dilatation which had accompanied the other responses at all levels appeared to increase in intensity and duration. Ventral to this area for 1.5 mm and immediately lateral to MMN all recorded autonomic responses faded completely and the intense pupillary response decreased rapidly but persisted in mild fashion to the point of deepest penetration in LMN. The stimulus was repeated as the electrode was raised now in 1 mm steps and all responses were consistent with those obtained during initial ventral penetration.

"<u>Wise</u>"

Penetration was precisely on the midline in the mid posterior portion of the MB's and at it's deepest point; the electrode tip rested in MMN about 0.5 mm from the bottom of the brain (figure 15).

Responses were first elicited 6.5 mm above the point of deepest penetration in the left wall of the third ventricle as in "Scratchy". Moderate pupillary dilatation accompanied by a mild increase in B.P. which followed stimulus onset by approximately 2 sec and had a duration of about 4 sec were the first responses observed at this level. One mm below this point, both responses increased in intensity and were accompanied by moderate muscle contractions, a pattern which persisted with little variation over an area 1 mm ventrad.

At the dorsal border of the mammillary bodies responses decreased as in previous animals, the decrease in pupillary dilatation being the least obvious. When the tip penetrated

Fig. 15. "Wise" Position of electrode tip at deepest penetration.

MMN, the only notable response remaining was that of pupillary dilatation. At this level, because of little response, current was increased from .8 to 1.6 ma and the pps increased from 100 to 300 (.3% - .9% time on), which increased the intensity and duration of the pupillary response and elicited a moderate increase in B.P. which occured however, only with a 5 sec latency and which did not subside for periods of 10 sec or more. It should be noted that the more normal B.P. response elicited from the area just dorsal to the mammillary bodies in this and previous animals usually appeared immediately following stimulus onset, and was of greater intensity and briefer duration.

As the tip was raised from it's deepest position (0.5 mm from the ventral border of MMN) the increased current (1.6ma) was maintained and the tissue stimulated at every 0.5 mm. All responses were slightly increased over their initial levels with .8 ma but the pattern of responding remained essentially unchanged. Strongest responses again occured approximately 2 mm dorsal to the mammillary bodies and decreased as the electrode was moved dorsad into the third ventricle. The electrode was then lowered again and the current increased further to approximately 2 ma. This resulted in a still greater strength of responses, particularly at those levels which

had been previously most sensitive to the stimulus.
In areas dorsal to the MB's, a sharp increase in B.P. immediately following the stimulus which did not return to normal for approximately 20 sec, a moderate increase followed by a slow decrease in respiration and an irregular heartbeat were noted. In addition, there was an increase in the duration of the pupillary response. As in a previous cat, some unusual eye movements were sometimes observed in response to stimulation of more ventral areas but this only occured when the stimulus was greatly increased over it's initial level. The response in this cat included turning of the eyes to one side simultaneous with a circular motion and pupillary dilatation. This pattern is different from the eye movements obtained from "Scratchy" and was also elicited from a slightly more dorsal area although the exact area stimulated is never certain, especially at increased stimulus levels when spread of current must be taken into consideration.

When the tip entered MMN for the second time, the sharp decrease in response again occured and the latency, particularly the B.P. response following the stimulus, again increased at deeper levels within the nucleus. Also at this point, very slight increases in respiration occured and persisted at the most ventral areas stimulated.

"Simba"

In this cat, the electrode tip at it's deepest point was exactly on the midline about 1 mm deep to the dorsal border of MMN in the mid posterior section of the mammillary bodies, while the barrel was always external to MMN (figure 16). The tip does not appear to have penetrated the cellular component of the nucleus because of it's precise midline location.

The first responses, a small pupillary dilatation and a very slight decrease in blood pressure which occured approximately 2 sec after stimulus onset, were observed 7.5 mm above this point in the area of the central medial nucleus of the thalamus. One mm deeper, the pupillary dilatation was stronger and a mild increase in respiration was noted immediately following the stimulus, while the B.P. response remained unchanged.

As in previous animals, the greatest response occured about 1 mm dorsal to the supramammillary decussation. Responses here included a moderate to strong decrease in B.P., an increase in respiration which included 2 shallow inspirations followed by a decrease in breathing rate and much deeper inspirations, a small increase in heartrate, and a full pupillary dilatation. Two mm deeper (just dorsal to the MB's) because there was little response, pulse duration was increased from .3 to .5 ms. This

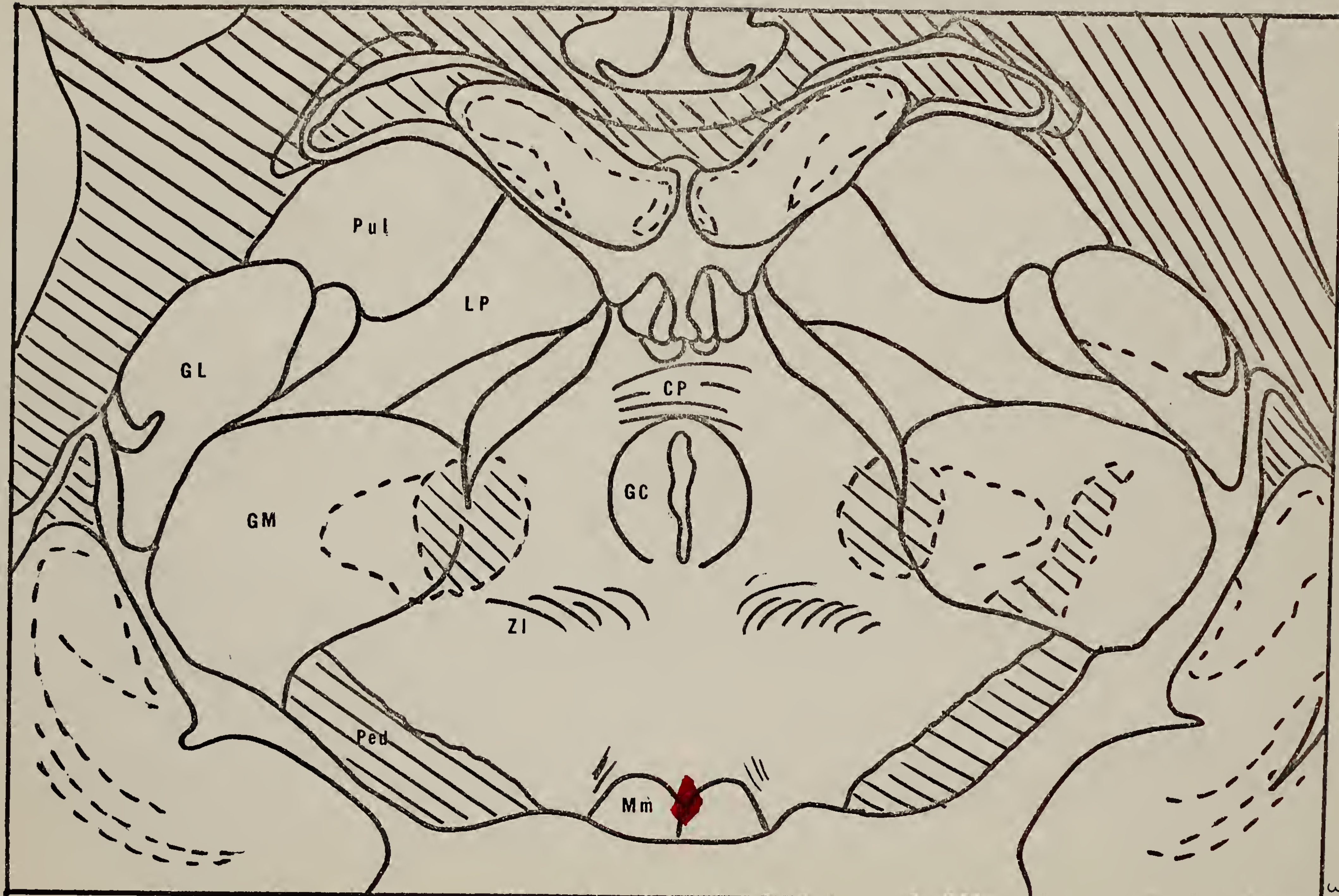

Fig. 16. "Simba" Position of electrode at deepest penetration.

increased the strength of response but the pattern remained the same with the exception of some muscular contractions which now appeared for the first time. In addition, the decrease in B.P. was preceeded by a mild increase which apparently was not detectable at lower stimulus levels.

One mm ventrad, when the tip reached the fiber capsule of the mammillary bodies, as in previous animals, there was a decrease in the strength of all responses. The initial increase in B.P. described above disappeared and the decrease which normally appeared after a brief delay was less. The respiratory response was also not as pronounced and the EKG pattern appeared irregular but showed no quantifiable change. One-half mm below this point (just inside the mammillary capsule) responses were barely detectable with the exception of a small pupillary dilatation and .5 mm deeper (at deepest penetration) only extremely slight responses could be detected other than the pupillary response. Current was now increased from 1 to 2 ma and stimulation yielded a stronger pupillary response but no notable increases in any other responses. The electrode was then raised in .5 mm increments and with 2 ma of current the responses obtained were very similar to those recorded on initial penetration.

"Wise,Jr."

In this cat the electrode tip was bent upon removal, having hit the skull just ventral to the MB's. Because of this, the tissue was quite torn in places and precise localization of responses was somewhat difficult. The electrode probably passed through the MMN just off the midline in the anterior portion of the MB's (figure 17).

The first stimulation was in the area of the supramammillary decussation. At this point there occured a very strong pupillary response, muscle contractions, a very strong increase in blood pressure and a moderate decrease in heartrate. In addition, the respiratory pattern changed from deep even breaths to several shallow rapid breaths followed by two longer and much deeper inspirations. At 1.5 mm lower (probably in the dorsal portion of MMN) a marked decrease occured in B.P. and heartrate (i.e. the increase in B.P. and the decrease in heartrate were not nearly as great as those recorded 1.5 mm above). The respiratory response however, remained prominent at this level. More ventral, throughout MMN, responses were only very slight except for a mild pupillary dilatation and at the deepest point (where the electrode probably hit the skull) only a very slight pupillary dilatation was detected even when the current was increased from 1 to 1.5 ma.

When the electrode was raised from it's deepest point, stimulation now elicited no responses except pupillary dilatation for 2.5 mm which was most likely the result of

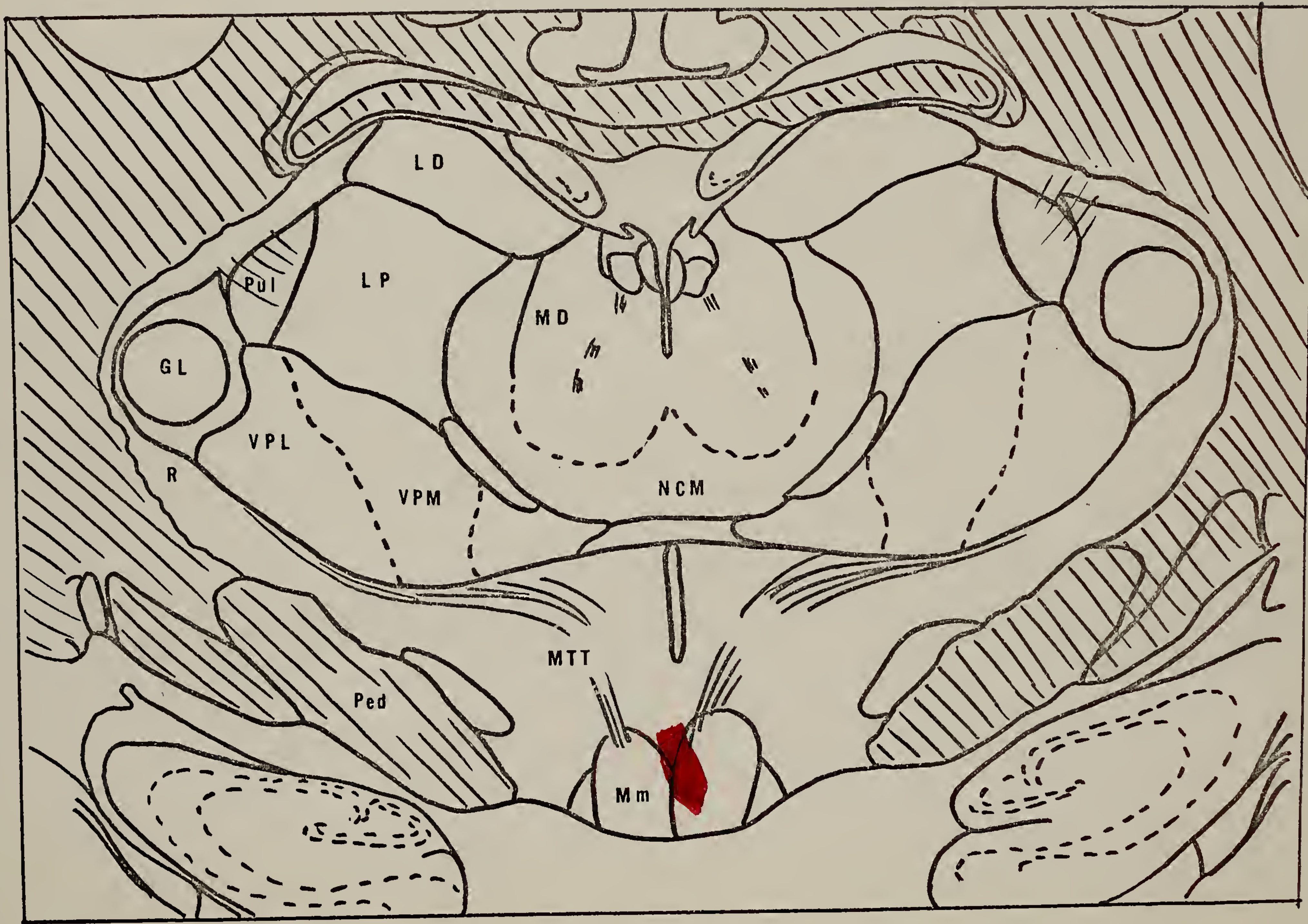

Fig. 17. "Wise, Jr." Position of electrode tip at deepest penetration.

the bent tip although no significant change in impedance occured. At approximately the dorsal border of the MB's, responses reappeared at moderate levels and followed a similar pattern to initial penetration. In addition, a striking localization of responses was found here with a strong pupillary dilatation, a moderate to strong increase in respiration and blood pressure and a moderate decrease in heartrate occuring, while 0.5 mm below this point no detectable responses were noted with the exception of a small pupillary dilatation. At more dorsal positions, responses obtained were similar to those recorded on initial penetration.

In four of the above animals, an electrode was placed in the medial portion of the hippocampus, while two animals had additional electrodes in the anteroventral and anteromedial nuclei of the thalamus. No autonomic responses were ever recorded from these structures over repeated stimulations.

<u>Conclusions</u>

It is apparent from the data that although some autonomic behavior can be elicited from electrical stimulation of the medial and lateral mammillary nuclei of the cat at current levels as low as 1 ma, in three of the five animals described, the only response obtained from ventral areas of MMN or LMN was that of pupillary dilatation. In some cases this response was only very slight and in all cases was less than the response obtained at more dorsal levels. In every animal, the point of greatest response was in the area of the supramammillary decussation, i.e. about 1.5 mm dorsal to the fiber capsule demarcating the dorsal border of the MB's. The extreme sensitivity of this particular area is believed to be caused by fibers of a ventral noradrenergic system coursing through the medulla and diencephalon (see Ungerstedt, 1971).

Although the intensity of the responses varied across subjects, in every case, a marked decrease in the rate of responding for that animal occured when the tip was lowered into the MB's, and in every animal continued to decrease as the electrode was moved to deeper levels. This specific pattern persisted even under conditions of increased current strength.

In two of the cats, slight to mild increases in B.P. and respiration did persist at ventral levels within MMN. These responses, however, did represent a decrease over those obtained at more dorsal points and for the most part, were only elicited under conditions of increased current. In addition, when autonomic responses did occur on stimulation of MMN, the delay between stimulus onset and the response was notably longer (usually 2 to 3 sec).

DISCUSSION

Although some involvement of the MB's in mediating autonomic functions cannot be entirely discounted by the data obtained, the contention that the MB's themselves serve as an autonomic "center" of the hypothalamus, highly sensitive to electrical stimulation, has certainly not been substantiated. Rather, it appears that the early results obtained by Ranson and Magoun (1939) of stimulation of the posterior hypothalamus and mammillary bodies, have essentially been replicated.

In spite of the fact that autonomic responses (particularly pupillary dilatation) were sometimes elicited from MMN, there was consistently a reduction in the strength of response when the electrode was passed into MMN from an area just dorsal. This reduction was not a gradual one which occured over several stimulations but usually appeared quite suddenly when the electrode was moved 1 mm or sometimes 0.5 mm ventral to the previous stimulation and may be attributed to a shunting of the electrical field by the myelinated fiber band of the mammillary capsule. It is also possible that the anesthetic used (sodium pentobarbitol), which did not seem to have any effect on responses elicited from more dorsal levels, somehow selectively inhibited responding in the MB's, but this does not seem likely.

The most persistent autonomic response obtained from mammillary areas was that of pupillary dilatation, which was consistently observed (although sometimes only as a very slight response) even when all other autonomic responses had faded to undetectable proportions. Current levels were never substantially lower than 1 ma and it must be assumed that the threshold for pupillary dilatation must be well below this value within the obviously broad geographical area from which the response was elicited.

Throughout stimulation of the MB's in all five cats, no significant qualitative differences in autonomic responses occured from those elicited at more dorsal levels, indicating that, at most, the MB's may be involved in autonomic functions very similar to but greatly reduced from those subserved by areas of the posterior hypothalamus, pre-rubral area, etc. One exception to this occured in two animals ("Scratchy"and"Wise")in which two different patterns of lateral eye movement were observed during stimulation of ventral portions of MMN. In both cases in which this unexpected response occured, electrode placement was in the posterior portion of the MB's. It seems possible that the response could be attributed to spread of current to the occulomotor nucleus of the mesencephalic tegmentum (1 to 2 mm posterior) or possibly even to the third cranial nerve which is located on the ventral surface of the brainstem approximately 2 mm caudal to the MB's. This nerve innervates four of the six extraocular muscles including the superior rectus, inferior oblique, inferior rectus, and medial rectus and is known to conduct impulses which produce elevation of the eyelid, vertical eye movements, converging eye movements, and also participate in conjugate horizontal eye movements (Truex and Carpenter, 1969).

The fact that all autonomic responses recorded from stimulation of the MB's appear greatly attenuated when compared to those obtained at more dorsal levels has been clearly established. Nevertheless, some autonomic responses were obtained when the electrode was clearly within MMN and this fact does imply MB involvement in such functions. Two possible interpretations will be discussed. First, it can be maintained that the cells of the MMN do not themselves result in autonomic responses when electrically stimulated. Rather, the responses elicited with the electrode in MMN are due to the firing of nearby cells outside the mammillary capsule. There is certainly some evidence to support this possibility. All responses throughout the study were long latency and never appeared sooner than 500 msec following stimulus onset. When electrodes were actually within the fiber capsule this latency increased to as much as 3 to 5 sec in some cases. When current strength was increased at this level, the responses also increased but the latency remained the same. In one instance ("Wise"), the latency of the B.P. response actually increased as the electrode was moved to deeper levels.

In one animal ("Simba"), the electrode tip did not actually penetrate the cells of MMN because it was located so precisely on the midline. In this case, the responses obtained were very similar to those recorded from other animals at similar vertical levels and included barely detectable respiration and B.P. changes and a mild pupillary dilatation. If these responses were the result of direct stimulation of cells within MMN, it seems reasonable to assume that significant differences in response strength would be detected when the tip was within the nucleus itself as opposed to being in a midline position between the two nuclei.

The second possible interpretation of the data is that cells of MMN actually do produce autonomic responses when stimulated. This could result from orthodromic firing over the MTT to ATN and cingulate cortex or to the limbic midbrain area of Nauta via the mammillotegmental tract. Autonomic responses have been elicited by stimulation of the cingulate cortex (although not from ATN in the present study) and the limbic midbrain area of Nauta is known to be strongly involved in autonomic functions. In addition, responses could be the result of an antidromic firing over the mammillary peduncle to the Gudden nuclei of the limbic midbrain area of Nauta. None of these possibilities seem improbable when the proposed function of the mammillothalamic system is reconsidered.

A mammal in his natural habitat, involved in initiating novel behaviors in response to dangerous situations must certainly undergo visceral changes relating to the decision to "freeze" or "flee". Indeed, it is quite unlikely that such a decision and the associated response could be made without considerable autonomic input, particularly relating to sympathetic types of functions. It is quite reasonable then, to attribute to the mammillary bodies a secondary autonomic function to provide necessary visceral input to a proposed phylogenetically recent, and apparently adaptive behavioral system.

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